

## EFFECT OF POLYHERBAL DRUG ON MENORRHAGIA AND ITS EVALUATION BY ASSESING BIOMARKER SERUM VEGF-A

MISHRA SARITA<sup>1</sup>, MUKTA SINHA<sup>2</sup>, PARAMESWARAPPA S. BYADGI<sup>3</sup> & S. P. MISHRA<sup>4</sup>

<sup>1</sup>Assistant Professor, Department of Prasuti Tantra, Jeevak Ayurvedic Medical College, Hospital & Research  
Center, Kamlapur, Akauni, Chandauli, Uttar Pradesh, India

<sup>2</sup>Professor, Department of Prasuti Tantra, Faculty of Ayurveda, Institute of Medical Sciences,  
Banaras Hindu University, Ajagara, Varanasi, Uttar Pradesh, India

<sup>3</sup>Associate Professor, Department of Vikriti Vigyan, Faculty of Ayurveda, Institute of Medical Science,  
Banaras Hindu University, Ajagara, Varanasi, Uttar Pradesh, India

<sup>4</sup>Head and Associate Professor, Department of Medical Biochemistry, Institute of Medical Sciences,  
Banaras Hindu University, Ajagara, Varanasi, Uttar Pradesh, India

### ABSTRACT

*Menorrhagia affect's woman's physical, emotional and social quality of life. In Ayurveda, Asrigdara is a disease, in which heavy menstrual bleeding is a main clinical feature, and now a days, it can be correlated with Dysfunctional Uterine Bleeding. The current modalities of treatment for the management of Dysfunctional uterine bleeding are only symptomatic and does not ensure permanent cure for the disease and is associated with their side effects and nothing to do with correction of basic pathology like hormonal regulation and disturbances of the uterine micro-environment. But, treatment described for Asrigdara in Ayurveda has multi-factorial approach, which corrects the basic pathology.*

### Aim of the Study

*To standardize a poly herbal combination drug (as per reference given in Ayurvedic text) preparation, which is probably acting on several level of pathogenesis, because of property of different ingredient of this combination, and its effect was evaluated by Biomarker Serum Vascular Endothelial Growth Factor-A, which get raised in case of Dysfunctional Uterine Bleeding (DUB) and regulate menorrhagia by various mechanisms.*

### Material and Methods

*The 120 women with menorrhagia were randomly selected and poly herbal combination of drug ( named Darvyadi Kashaya-Extract of eight herbal drugs in water, decoction )was given alone per oral, along with intrauterine instillation (Uttar Basti) of oil, processed by water extract of same eight drugs. Patients were divided into three groups, 40 patients in each and were given the oral drug alone, intrauterine instillation of the oil alone and oral drug with intrauterine instillation of the oil. Treatment was given orally for three months, 20 ml of decoction in twice daily dose with honey and intra-uterine instillation (3-5 ml of oil in increasing order) was given for three day in each month, after clearance of menses for three month. Result was assessed on Duration, Inter-menstrual Period and Amount of menstrual blood by the appropriate scoring, and serum VEGF-A was measured by ELISA method, for which venous blood was collected during proliferative phase before and after treatment.*

**Result**

*Improvement in symptoms were statistically significant in all the three groups along with reduction in Serum VEGF-A, but in inter group comparison, improvement in the patients who had taken oral drug along with intrauterine instillation was more i.e. in Trial group C.*

**Conclusion**

*Poly herbal combination of drug (named Darvyadi Kashaya) along with intrauterine instillation (Uttar Basti) of oil improves the symptoms related to menorrhagia related to Dysfunctional Uterine Bleeding (DUB). It can be used as an effective alternative of hormonal treatment and other measures used to control menorrhagia.*

**KEYWORDS:** Ayurveda, Asrigdara, Angiogenesis, Serum Vascular Endothelial Growth Factor –A (Vegf-A), Darvyadi Kashaya & Darvyadi Tail Uttar Basti

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## INTRODUCTION – OBJECTIVE AND BACKGROUND

In *Ayurveda*, different types of drug and treatment modalities are described for heavy bleeding during menses (*Asrigdara*), among them, *uttar basti* is described as best for gynecological problems. So, in present study, *Darvyadi Kashaya* (Decoction made up of eight herbs i.e. *Daruharidra* (*Berberis aristata*), *Rasanjana* (extract of *Berberis aristata* in milk- *Berberis aristata* was boiled with milk, till it became solid in consistency), *Kiratatikta* (*Swertia chirata*), *Mustak* (*Cyperus rotundus*), *Bilva* (*Aegle marmelos*), *Arka* (*Calotropis procera*), *Vasa* (*Adhatoda vasica*) and *Chandana* (*Pterocarpus santalinus*) and *Darvyadi Tail*, prepared with Sesame oil (*Sesamum indicum*) processed with a decoction and paste of these drugs was selected and given in the form of *Uttar Basti* ( *intrauterine instillation of oil* ). In *Ayurvedic* text named '*Bhav Prakash Samhita [ Bhav Mishra ]*', there is only reference of *Darvyadi* Decoction to treat heavy menstrual bleeding, but keeping the concept of *uttar basti* to treat gynecological diseases in the best way [ *Sushruta*], the oil processed with the same decoction was also selected alone, along with decoction for comparative study. For standardization of the effect of these drugs, biomarker serum VEGF-A was selected because of its extensive role (on vasodilatation, vascular fragility and permeability) in pathogenesis of heavy menstrual bleeding in DUB .

## MATERIAL AND METHODS

### Selection of Cases

Patients attending to the outpatient department of Prasuti Tantra S.S. Hospital, B.H.U., Varanasi were randomly selected. The cases selected were having complaints of excessive and/or prolonged blood loss, during menstruation or short inter-menstrual period.

### Criteria for Inclusion

- Married women of reproductive age group, with complaints of excessive bleeding per vagina during menstruation, either in amount or in duration or both or short inter menstrual period for 3 consecutive menstrual cycles were selected.
- Patients, who are neither using oral contraceptive pills nor IUCD for contraception or hormonal treatment.

- The patients of reproductive age were included in the study, irrespective of obstetric history related to parity.
- The patients who were ready for consent, necessary investigations and agreed to come for follow up regularly selected.
- Infections such as candidiasis, Trichomoniasis or any other form of vulvovaginitis and pelvic congestion were initially treated with respective drugs, and when symptoms and signs totally disappeared, then they were included in the study.

#### **Criteria for Exclusion: Criteria for Exclusion**

Associated with any currently ongoing research study, Unmarried, Postmenopausal, Recent delivery or abortion, Patient using hormonal preparations, Patient having any organic pathology e.g. Cervical erosion, Cervical or uterine polyp, fibroid uterus, adenomyosis, PID, carcinoma cervix, carcinoma uterus etc. Intrauterine device in utero, any systemic diseases e.g. Cardiac disease, Thyroid disorders, Hypertension, Kidney diseases, Tuberculosis and STDs etc. Any allergy to the drugs, Severe anemia, Jaundice and Psychiatric patient.

#### **Grouping of Cases**

In the present study, assessment of said compounds in Asrigdara was studied in 120 patients, dividing into three groups A, B, C respectively.

**Groups A (Control Group):** Patients were selected and administered *Darvyadi Kashay* Orally.

**Group B (Trial Group):** Patients were selected and administered *Darvyadi Tail Uttar Basti* **intrauterine**.

**Group C (Trial Group):** Patients were selected and administered *Darvyadi Kashay* Orally along with *Darvyadi Tail Uttar Basti* **intrauterine**.

#### **Doses**

- *Kashaya* -20 ml twice a day with 10 ml honey
  - Duration: 3 months
  - Route of administration: Oral
- *Basti*- 3-5ml ( in increasing order) for three days in a month, after clearance of menses

Duration: 3 months

Route of administration: Vaginal

#### **Follow Up Study**

Total four follow ups were done. The cases were followed at monthly interval for 3 months with treatment after clearance of menses, and one last follow up was done after month after clearance of menses without drug, so as to note any recurrence of symptoms.

**History**

The detailed history of the present complaints, with duration and associated symptoms was taken on pre-designed specific proforma. Detailed interrogation regarding present symptoms, especially the amount of blood loss, duration and interval of menses and other associated symptoms were taken.

History of past illness(if any) including diabetes mellitus, hypertension, Tuberculosis, Jaundice, Bronchial Asthma, Surgical intervention, Blood transfusion, drug sensitivity were noted. Personal history regarding habits, previous menstrual history, and obstetric history, including psychological upset, mania and psychosis was recorded. Family history was also noted. Other important points like marital status, status of hygiene, personal history and socioeconomic status were also noted.

**Clinical Examinations****General Examination**

General condition of the patient, Pulse, Temperature, Blood pressure, Pallor, Icterus, Clubbing, Cyanosis, Edema, Lymphadenopathy etc. noted.

**Systemic Examination**

Examination of central nervous system, gastrointestinal tract, respiratory system, cardiovascular system, urogenital systems were done.

**Local Examination**

Local examination of reproductive system was done thoroughly. The condition of the vulva and vagina was noted. P/S (per speculum) examination was done to know the condition of the cervix for congestion, hypertrophy, erosion, ectropion, entropion, nebothian follicles, any discharges (It's amount, color, odor and consistency). Per vaginal examination was done to know the size, shape, direction, mobility and consistency of the uterus with the condition of the adnexae and the observations were recorded.

**Investigations Advised**

After detailed history and complete examinations, all the cases were subjected to following investigations before the trial.

**Blood Examination**

Hemoglobin percentage by Sahli's method, Total leukocytes count by Neubauer's Chamber method, Differential leukocytes count, Erythrocyte sedimentation rate - Westergren method, Bleeding time - Dukes method, Clotting time - Capillary tube method, Platelet count - by indirect method, Prothrombine Time, APTT, LFT, Sr. Creatinine, HIV, VDRL, Thyroid profile, FBS.

- **Urine Examination:** For routine and microscopic examination. For culture and sensitivity test (if needed)
- **Stool Examination :** for ova and cyst
- **Ultra Sonography (USG):** for condition of uterus and adnaexae, any pelvic pathology and thickness of endometrium (ET)

- **Pap smear** (if needed)
- **High Vaginal Swab for Culture Sensitivity** (if needed)
- **Endometrial Biopsy** (if needed, in patients having age >35 yrs)

#### **Criteria of Assessment**

Scoring of the symptoms was done before and after the study, purely on the basis of patient's explanations.

#### **Duration of Menstrual Bleeding**

Duration of bleeding was categorized according to bleeding occurring in total number of days. This was noted as per the patient's explanations.

#### **Inter-Menstrual Period**

The number of days in between two consecutive menstrual cycles was counted.

#### **Amount of Blood Loss**

It was assessed on the basis of statements given by the patient. All the patients were advised to use a standard size pad "Stay Free secure" (Johnson & Johnson) having average pad weight 12.5 gm, asked to change the pad, when it is fully soaked and note the count of the pad used per day during menstruation. Hence, the number of pads used in every cycle recorded.

**Table 1: Showing the Parameters which were Graded in the Study**

<b>Symptoms</b>	<b>Criteria</b>	<b>Score</b>
Amount of bleeding per menstrual cycle	Complete soakage of 2-3 pad in 24 hours(Average)	1
	Complete soakage of 4-5 pad in 24 hours (Moderately excessive)	2
	Complete soakage of >6 pad in 24 hours (Excessive)	3
Duration of menstrual Bleeding	Bleeding for 2-3 days (Normal)	1
	4-5 days (Moderately prolonged)	2
	More than 6 days ( prolonged)	3
Inter menstrual Period	15-20 days (Very short)	1
	2 1-25 days (Short)	2
	26-30 days (Normal)	3

#### **Associated Symptoms**

Pain in lower abdomen, Backache, Bodyache, Headache, Pain in the calf muscle, Breast tenderness, Giddiness, Fever, Burning in feet & palm, nausea, Vomiting, Loose motion, Anxiety, Weakness, Loss of appetite was noted during each menstrual cycle, grading was done, score 1 was given for the presence of symptom and score 2 was given for the absence of symptom.

Table 2: Showing the Grouping of Patients According To Treatment

S. No	Groups	Drug	Rout of Administration	Dose	Duration
1.	Group A (Control Group)	<i>Darvyadi Kashay</i> with Honey	Oral	20 ml twice a day	3 months
2.	Group B (Trial Group)	<i>Darvyadi Tail Uttar Basti</i>	intra uterine.	3-5ml (in increasing order)	for three days in a month after clearance of menses for three cycles
3.	Group C (Trial Group)	<i>Darvyadi Kashay</i> along with <i>Darvyadi Tail Uttar Basti</i>	Oral+ intra uterine.	20 ml twice a day + 3-5ml (in increasing order)	3 months + for three days in a month after clearance of menses for three cycles

### Observations

The observations of the patients of all the three groups are discussed further on the basis of history (age, gravidity, parity, marital status, chief complaints with duration, personal history etc.), physical examination, local examination, investigations and different parameters like the duration of bleeding, inter menstrual period, amount of bleeding and associated symptoms.

### Follow Up

Patient of both group were followed up at regular interval of one month (after bleeding is complete) for the evaluation of drug on present complaints and associated complaints. Three follow up study were done, while the patient was on drugs (then the drug was withdrawn) and fourth follow up was taken without drug to check the efficacy of treatment and to know the status of symptoms of the patients. Specific scoring of duration of bleeding, intermenstrual period, amount of bleeding, consistency of menstrual blood and change in associated symptoms were also recorded.

### Sample Collection for Estimation of Serum VEGF-A

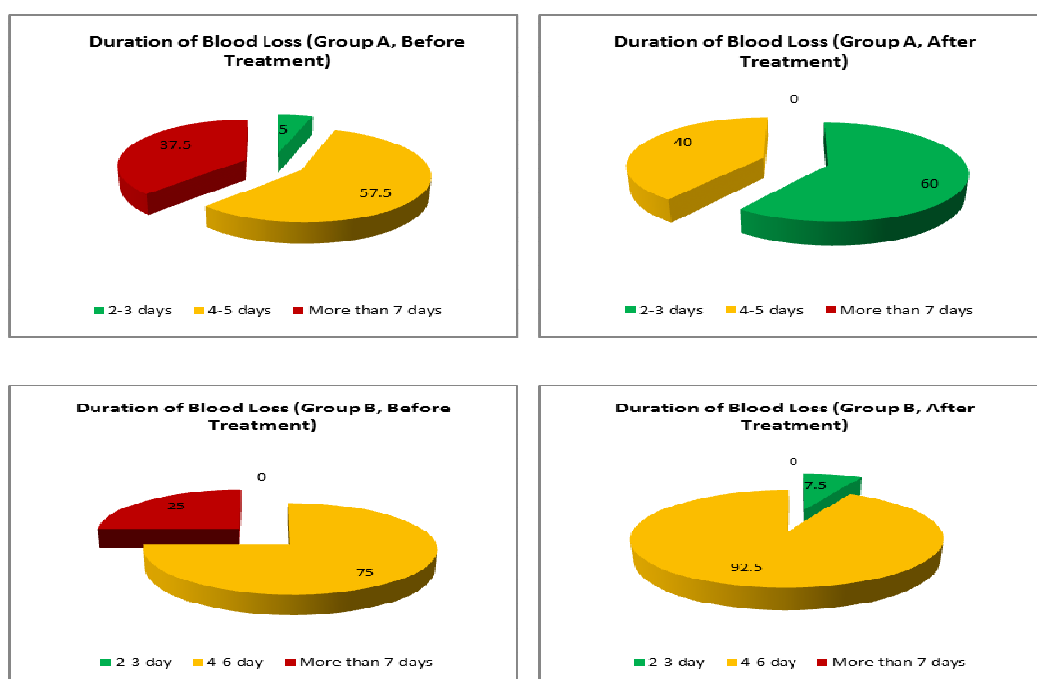
2ml of venous blood was withdrawn from selected patients after proper consent in their early- mid proliferative phase. Blood was centrifuged to separate serum and serum was stored in plain vial after proper labeling and kept in deep freezer. The sample was collected before and after treatment. After collection of required number of samples, ELISA Assay was done by Ray Bio Human VEGF-A ELISA kit in the department of Biochemistry, Institute of Medical Sciences, BHU, Varanasi.

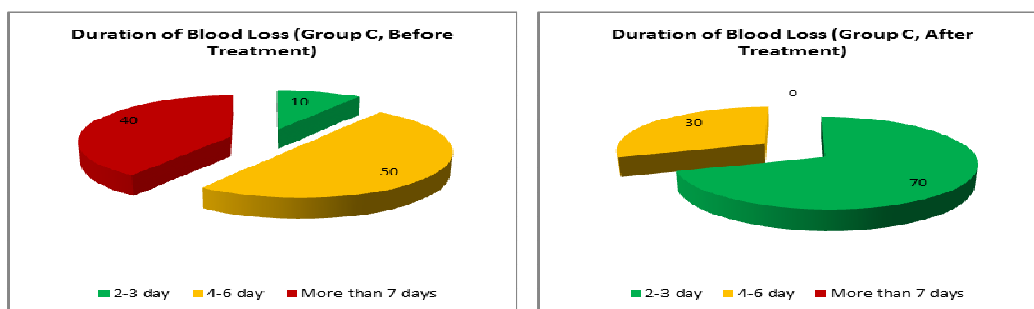
### Method of the Estimation of Serum VEGF-A by ELISA Kit

(RayBio Lot # 0828150196 ) – This kit is an in vitro enzyme linked immune sorbent assay for the quantitative measurement of human VEGF in serum, plasma and cell culture supernatants. This assay employs on antibody specific for human VEGF coated on wells in ELISA plate. Standards and sample were pipetted into the wells, and the VEGF present in a sample was bound to the wells by the immobilized antibody. HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color developed in proportion to the amount of VEGF bound. The stop solution changed the color from blue to yellow, and the color was measured at 450nm by the ELISA plate reader.

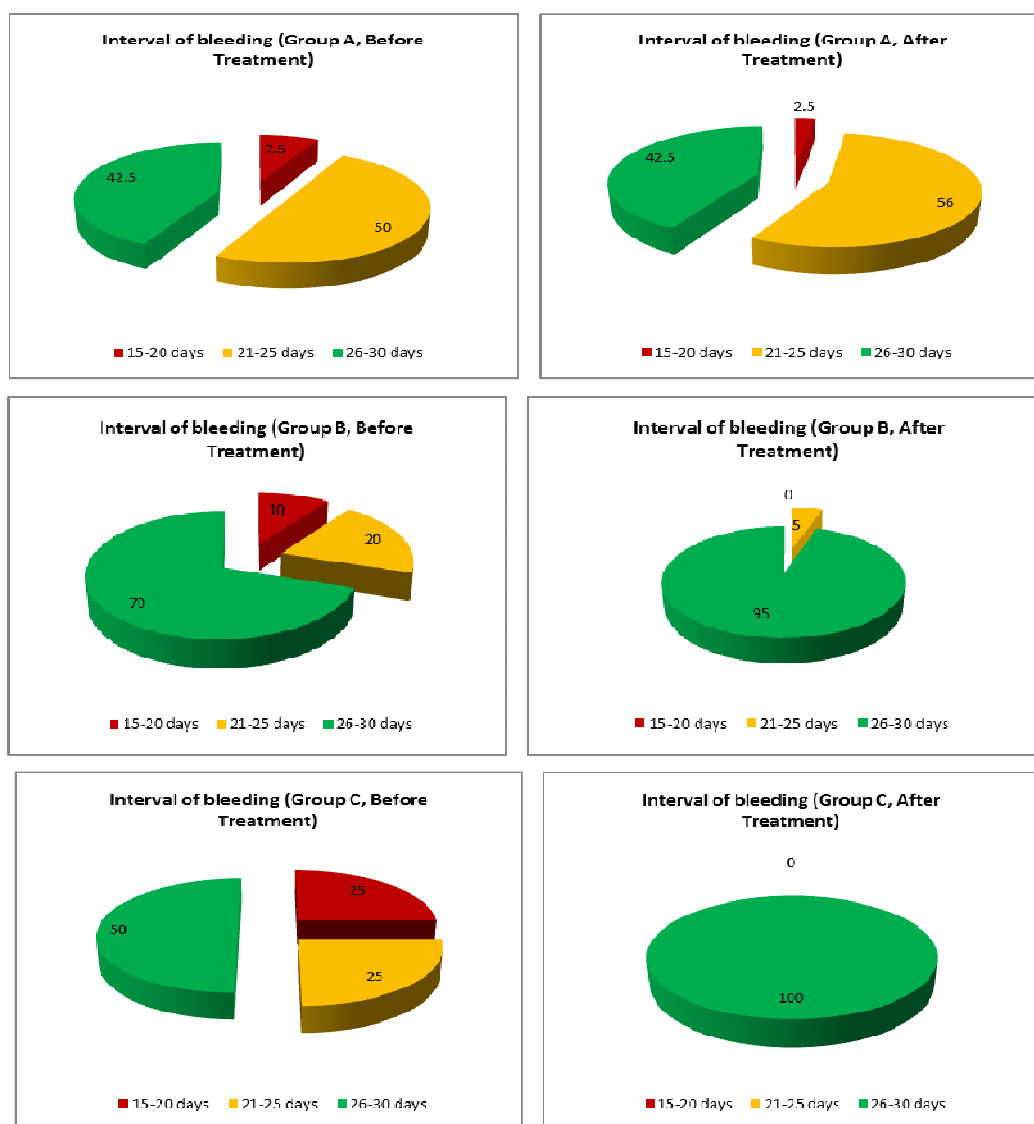
- 100 µl of each standard and sample were added into appropriate wells. Wells were covered and incubated for 2.5 hours at room temperature.
- After 2.5 hours, the solution was discarded and the wells were washed 4 times with 1 x wash solution. Washing was done by filling each well with wash buffer (300 µl) using a Multi Channel pipette. Complete removal of liquid at each step was done for good performance. After the last wash, the plate was inverted and blotted against clean paper towel.
- 100 µl of 1x prepared biotinylated antibody was added to each well and the plate was again incubated for 1 hour at room temperature.
- After 1 hour, solution was again discarded and 4 times washing was again done with 1x wash solution.
- 100µl of the prepared streptavidin solution was added to each well, again the plate was incubated for 45 minutes at room temperature with gentle shaking.
- After 45 minutes, again solution was discarded and 4 times washing of the ELISA plate was done by 1x wash solution.
- 100µl of TMB one step substrate reagent (item H) was added to each well, and the plate was incubated for 30 minutes at room temperature in dark with gentle shaking.
- After 30 minutes, 50µl of stop solution (Item I) was added to each well, and ELISA plate was read at 450nm to measure respective optical density of each well by the ELISA plate reader.

## RESULTS





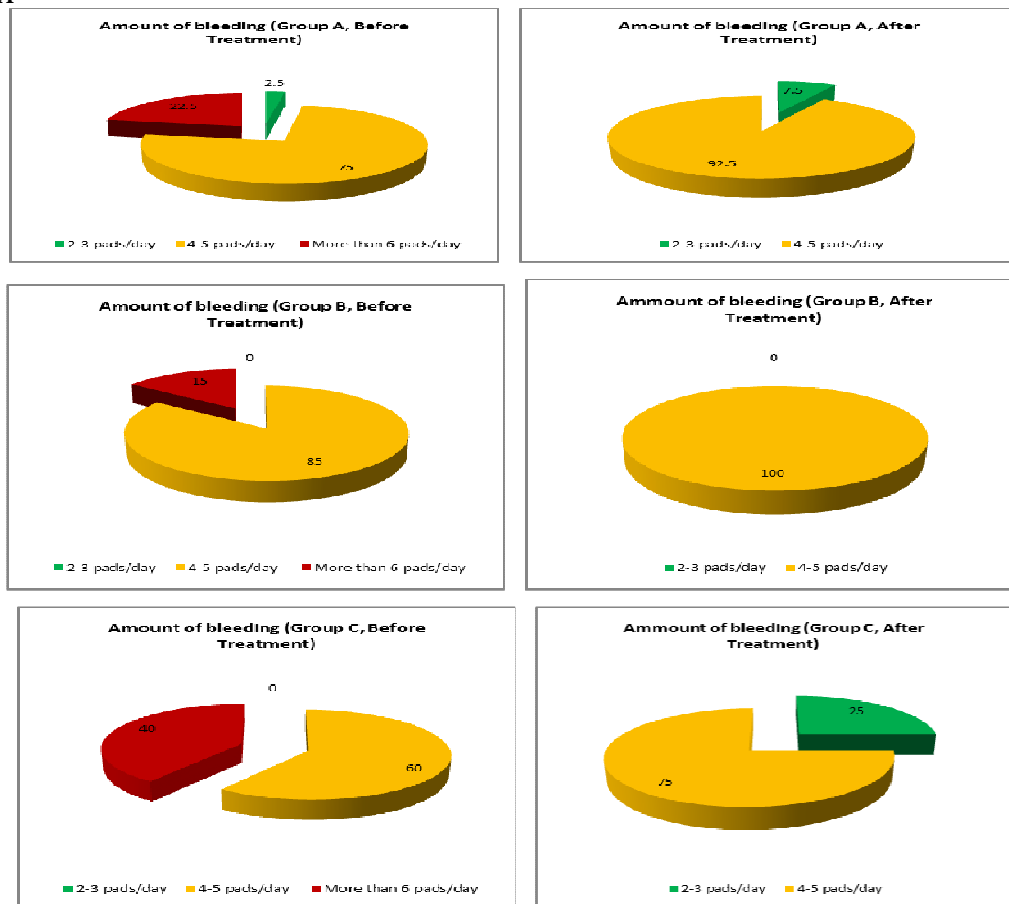
**Graphs 1: Showing Improvement in the Duration of Menstrual Bleeding after Treatment in all the Three Groups**



**Graphs 2: Showing improvement in Interval of Menstrual Bleeding after Treatment in all the Three Groups**



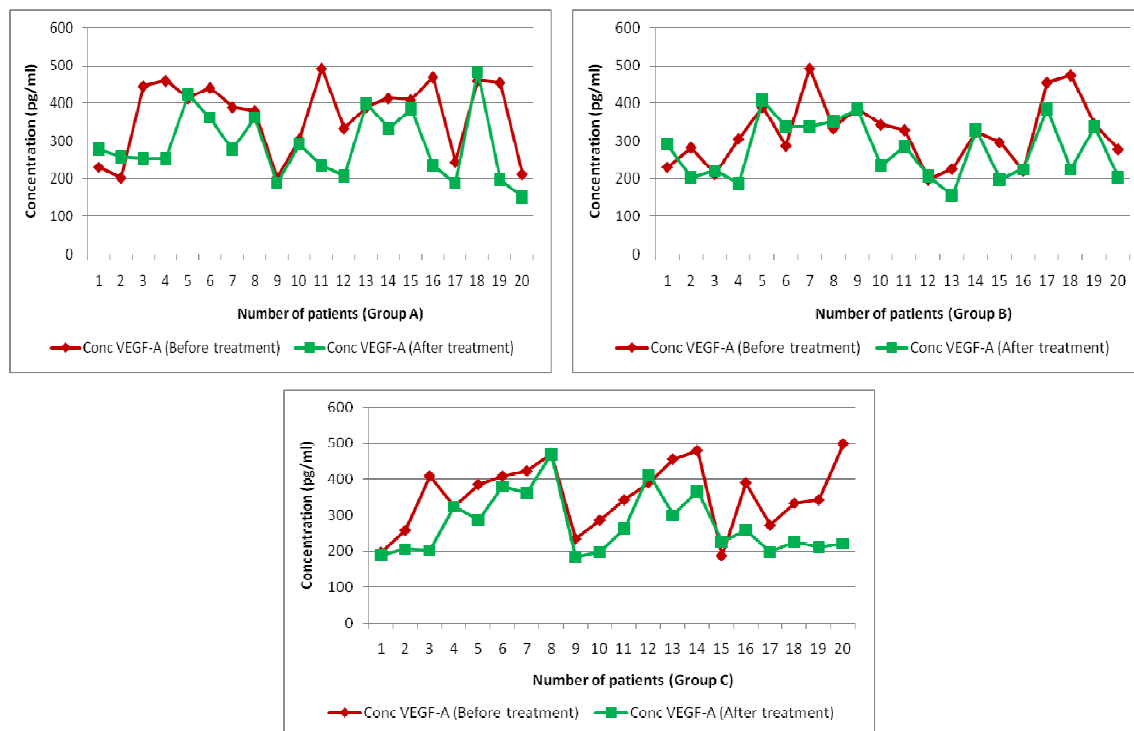
**Effect of Polyherbal Drug on Menorrhagia and  
Its Evaluation by Assessing Biomarker  
Serum VEGF-A**



**Graphs 3: Showing Improvement in the Amount of Menstrual Bleeding after treatment in all the three Groups**

**Table 3: Showing the incidence of Serum Vascular Endothelial Growth Factor-A (VEGF – A) in all the Three Groups**

Group	Concentration of VEGF –A (in pg/ml) Mean +- SD		Within the Group Comparison BT- AT
	Before Treatment	After Treatment	
Group A	367.5+- 99.55	287.92+- 90.11	79.63+- 101.97 t=3.49 p=.003
Group B	320.10+-86.34	275.47+-78.21	44.63+- 76.94 t= 2.59 p= .018
Group C	354.39+- 92.53	274.06+- 85.12	80.30+- 77.85 t= 4.61 p= <.001



**Graph 4: Showing Improvement in Serum VEGF-A after Treatment in all the Three Groups**

So, it can be said that raised concentration of serum VEGF-A is a cumulative expression of various pathological events, those are going on at various level of endometrium, and body and reduction of its concentration shows that the drug has acted on those pathological factors.

Results show that serum VEGF-A level gets reduced in all the three groups which were statistically significant, but the improvement was more ( $p > .001$ ) in trial group C, in which patients took oral drug with *uttar basti*.

## DISCUSSIONS

Heavy, prolonged and frequent bleeding is often considered as dysfunctional uterine bleeding (DUB), which is devoid of any pregnancy causation or systemic disease, hormonal disturbance is considered to be one of the causes of excess endometrial proliferation (Kenneth M. et al., 2001) that rooted in capillaries and small vessels surrounding endometrium (Hickey M, 2006).

The complex mechanism in human endometrium in each menstrual cycle requires an endothelial cell specific angiogenic peptide that orchestrates vascular and glandular proliferation, differentiation and regeneration in order to prepare implantation of an embryo. VEGF and other angiogenic protein appear to play a fundamental role in both physiological and pathological neovascularisation. The synthesis of new blood vessels depends solely on the interaction between different growth factors and hormones. Several growth factors like, transforming growth factor (TGF-beta) and vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) have been found to have a direct effect on endometrial angiogenesis (G. guset, et al., 2010) and vascular fragility, any disturbance in which causes heavy bleeding during menses.

There are several angiogenic growth factors in the human endometrium, which are responsible for the endometrial vascular integrity and permeability, and any disturbance in these causes bleeding, as in case of DUB.

The most celebrated actions of VEGF-A are in endothelial cells, inducing proliferation and migration.

A possible additional effect of VEGF-A on the vasculature is that of vasodilatation. VEGF-A stimulates nitric oxide release from endothelial cells. VEGF-A also induces the release of prostacyclin, another vasodilating agent, by activating cytosolic phospholipase A2, causing the release of arachidonic acid. Both of these effects could modulate the contractility of the spiral arterioles, and thus alter the volume of menstrual blood loss.

In addition to its actions on the endothelial cells, VEGF-A has important effects on serine proteinases which influence coagulation. This is important in the context of the endometrium, as coagulation (Gleeson, et al., 1993) here differs from that in other regions of the body, in that, rapid coagulation and fibrinolysis of the blood occurs as it passes down the spiral arterioles. This is presently thought to be an important part of the mechanism of excessive menstrual bleeding. Elevated levels of tissue plasminogen activator (tPA) are found in endometrium of women with heavy periods and drugs, which reduce the degradation of fibrin platelet clots to fibrin degradation products, reducing menstrual blood loss by about 50% (Cameron, et al., 1995).

## **INCREASED ENDOMETRIAL VASCULAR FRAGILITY**

It is regulated by-

- Hormonal regulation
- Inflammatory regulation
- Oxidative Stress

### **Hormonal Regulation**

Due to hormonal imbalance (unopposed estrogen in case of DUB) at the level of uterine endometrium, there is increased dilatation of the arterial supply in the endometrium and lead to proliferation of endometrium, and would associate with abnormal thickening of the endometrium without proper architectural integrity.

Large, thin-walled, tortuous, superficial endometrial vessels often can be demonstrated on the surface of the endometrial hyperplasia; increase of blood loss is due to fragility of blood vessels. Vascular tone is reduced by an unopposed estrogen and has a direct effect by inhibiting vasopressin release, that causes vasodilatation and increase blood flow (Springer-Verlag; 1985).

When estrogen synthesis is uneven and unopposed, synthesis of prostaglandin (PG) in endometrium would be less, and in this condition synthesis of prostaglandin E is higher than PGF.

### **Inflammatory Regulation**

Multiple mechanisms involve in the accuracy of the angiogenic balance between angiogenic factors and interaction with a compound of extracellular matrix (ECM ).

ECM is considered to be the storage place for angiogenic stimulator and inhibitors. These molecules are found to bind to component of ECM and they have been released via cleavage by protease, i.e. matrix metalloproteases (MMPs),

which proteolytically cleave and activate precursors of angiogenesis promoters. One of the compounds that growth factors bind with, is a heparin sulfate proteoglycans in the extracellular matrix. MMPs generate growth-promoting signals by releasing growth factors bound to them, and causing a generation of activating ligands for integrin signaling (Fraser IS, et al., 1996).

Among all the growth factors, vascular endothelial growth factor (VEGF), is considered to be a potent and best studied angiogenic peptide that is released from ECM by the proteolytic action of MMPs and plasmin.

The activity of MMPs on angiogenic growth factors, chemokines, growth factor receptors, apoptosis mediators, adhesion molecules is critical for the rapid cellular responses, and essential for angiogenesis and also involved in mediating tumor growth and progression.

VEGF is regulated by a variety of stimuli such as hypoxia, growth factors, exogenous growth factor stimulator, transformation, mutation, estrogen, TSH(thyroid-stimulating hormone), tumor promoters and NO (nitric oxide) (Agrez M, et al., 1994). VEGF contributes to vascular permeability provokes dilatation and promote angiogenesis

### **Oxidative Stress and the Endometrial Cycle**

Aerobic metabolism is associated with the generation of pro-oxidant molecules called free radicals, or reactive oxygen species (ROS) that include the hydroxyl radicals, superoxide anion, hydrogen peroxide, and nitric oxide. There is a complex interaction of the pro-oxidants and antioxidants, resulting in the maintenance of the intracellular homeostasis. Whenever there is an imbalance between the pro-oxidants and antioxidants, a state of oxidative stress (OS) is initiated. OS is involved in the modulation of cyclical changes in the endometrium. Fluctuations in the expression of SOD in the endometrium have been investigated. Altered SOD and ROS levels have been demonstrated in the endometrium during the late secretory phase, just before menstruation. An elevated lipid peroxide concentration and decreased SOD concentrations have been reported in human endometrium in the late secretory phase, and these changes may be responsible for the breakdown of the endometrium, implicating the involvement of OS in the process of menstruation (Lee S., et al., 2005). The expression of endothelial NO synthase (NOS) and inducible NOS have been demonstrated in the human endometrium and the endometrial vessels (Sugino et al., 2004). Endothelial NOS is distributed in glandular surface epithelial cells in the human endometrium. NO is thought to regulate the microvasculature of the endometrium. Expression of endothelial NOS mRNA has been detected in the mid secretory phase and late-secretory phase, indicating its involvement in the decidualization of the endometrium and menstruation. Endothelial NOS is also thought to bring about changes that prepare the endometrium for implantation. Recent studies exploring the underlying mechanisms of endometrial shedding have established that, estrogen and progesterone withdrawal in endometrial cells cultured in vitro leads to a decrease in SOD activity, thereby increasing ROS concentrations.

In turn, ROS may activate nuclear factor kappa, which stimulates increased cyclooxygenase-2 mRNA expression and prostaglandinF2 $\alpha$  synthesis, facilitating the physiological changes required for endometrial shedding and/or implantation to occur.

VEGF and Ang-2 are key regulators of endometrial angiogenesis.

VEGF and Ang-2 are induced by hypoxia and ROS (Rosselli M, et al., 1998) and have been observed to be unregulated in the endometrium of patients taking long-term progestin-only contraceptives. The changes in VEGF and Ang-2 expression are thought to play an integral role in producing the abnormally distended, fragile vessels that are the

cause of abnormal uterine bleeding associated with long-term progestin-only contraceptive use. The later induces the abnormal angiogenesis by decreasing endometrial blood flow, inducing hypoxia ( Park JK, *et al.*, 2006).

### **Role of VEGF in Menorrhagia**

The observation that vascular repair is an obvious feature of menstruation does not mean that disturbance of this mechanism cause menorrhagia, but that there is other evidence which suggests that altered VEGF-A expression may be involved. To understand this, the multiple actions of VEGF-A need to be considered and placed within the context of the mechanisms of menstruation.

The two hypotheses currently used to explain heavy periods suggest either increased fibrinolytic activity, or enhanced vasodilatation. In the former circumstance, this is suggested by the finding of reduced platelet fibrin plug formation in menstrual endometrium compared with other parts of the body (Christiaens *et al.*, 1980), the presence of higher tPA concentrations in endometrium of women with objective menorrhagia (Sheppard, 1996), and the observation that Tranexamic acid, which impairs fibrinolysis, reduces menstrual blood loss by about 50%. VEGF stimulates tPA synthesis by endothelial cells and increases vascular permeability, resulting in activation of the extravascular coagulation cascade with the consumption of fibrin (Pepper *et al.*, 1991). The release of VEGF by activated platelets provides a further means by which VEGF increases tPA release, as menstrual platelets are activated in the uterine cavity (Rees *et al.*, 1984). These observations are particularly important, as they provide an explanation which bridges the vascular theories of menstruation with those of the coagulation system.

Pilot information suggests that, immune reactivity for VEGF-A is increased in women with menorrhagia. The potent vasodilator, nitric oxide, is released by endometrium (Tseng *et al.*, 1996) and VEGF stimulates NO release from endothelial cells (Papapetropoulos *et al.*, 1997). With the increased release of the vasodilatory PGI<sub>2</sub>, both of these actions would be expected to promote vasodilatation.

The recent finding that the proliferative activity of endothelial cells is doubled in endometrium of women with heavy periods (Kooy *et al.*, 1996) further suggests that, disturbances of angiogenic growth factor expression or function could be involved in the pathology of menorrhagia. Increased levels of VEGF are found in the peritoneum of women suffering from endometriosis (Shifren *et al.*, 1996), which may provide a further link between menorrhagia and the establishment of ectopic endometrial explants. The close relationship between VEGF and the coagulation system and its possible effects on vascular tone further implicate disorders of VEGF expression with common pathologies of the female reproductive tract (Kooy *et al.*, 1996).

## **RESULTS**

It shows that, serum VEGF-A level get reduced in all the three groups which were statistically significant, but the improvement was more in trial group C (p value >.001), in which patients took oral drug with *uttar basti*.

## **CONCLUSIONS**

*Darvyadi* decoction and *uttar basti* with *Darvyadi* oil can be helpful in managing heavy bleeding related to DUB effectively, that is evidenced by reduced levels of biochemical marker VEGF-A. It can be use as a better alternative treatment for DUB.

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